MycoKeys 51: 97–106 (2019)
doi: 10.3897/mycokeys.51.34956
http://mycokeys.pensoft.net

RESEARCH ARTICLE

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Erysiphe deutziicola sp. nov. (Erysiphaceae, Ascomycota), a powdery mildew species found on *Deutzia parviflora* (Hydrangeaceae) with unusual appendages

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Academic editor: *N. Wijayawardene* | Received 30 March 2019 | Accepted 4 April 2019 | Published 8 May 2019

Citation: Qiu P-L, Braun U, Li Y, Liu S-Y (2019) *Erysiphe deutziicola* sp. nov. (Erysiphaceae, Ascomycota), a powdery mildew species found on *Deutzia parviflora* (Hydrangeaceae) with unusual appendages. MycoKeys 51: 97–106. https://doi.org/10.3897/mycokeys.51.34956

Abstract

A powdery mildew (Erysiphales) has recently been collected on leaves of an ornamental shrub *Deutzia parviflora* in Baihua Mountain, Beijing, China. Microscopic examination of the chasmothecia suggested a species belonging to *Erysiphe* sect. *Erysiphe*, above all due to mycelioid chasmothecial appendages, although circinate apices of the appendages were rather in favour of *Erysiphe* sect. *Uncinula*, which is a fairly rare combination of appendage characteristics in *Erysiphe*. Phylogenetic analyses of ITS and 28S rDNA sequences demonstrated that the two examined powdery mildew collections on *D. parviflora* clustered together as an independent lineage within *Erysiphe* with 100% bootstrap support, representing a species of its own, which is phylogenetically allied to, but clearly distinct from *Erysiphe deutziae* and, in addition, morphologically quite different from all known *Erysiphe* species on hosts belonging to the Hydrangeaceae. The new species on *D. parviflora* is described as *Erysiphe deutziicola*.

Keywords

Erysiphales, powdery mildew, pathogen, ITS, 28S rDNA, phylogeny

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Introduction

The family Hydrangeaceae comprises 17 genera and about 220 species distributed in temperate and subtropical regions of the Americas, Pacific islands, Asia and Europe (Kubitzki 2004). One of the largest genera, *Deutzia*, includes important ornamentals and is known to be used to treat enuresis, malaria and scabies in China (He 1990). Amongst the genera of Hydrangeaceae, *Deutzia*, *Hydrangea*, *Schizophragma*, *Jamesia* and *Philadelphus* have been reported as hosts of powdery mildews (Braun and Cook 2012). Nine species are currently known on hosts of these genera, viz., *Erysiphe deutziae* (Bunkina) U. Braun & S. Takam. on *Deutzia*, *E. hydrangeae* (Z.X. Chen & R.X. Gao) U. Braun & S. Takam. on *Hydrangea*, *E. poeltii* U. Braun on *Hydrangea*, *E. schizophragmatis* (Tanda & Y. Nomura) U. Braun & S. Takam. on *Hydrangea* and *Schizophragma*, *E. yanshanensis* T.Z. Liu & U. Braun on *Hydrangea*, *Golovinomyces orontii* on *Hydrangea*, *Phyllactinia jamesiae* U. Braun on *Jamesia*, *P. philadelphi* (Jacz.) Bunkina on *Philadelphus* and *Pseudoidium hortensiae* (Jørst.) U. Braun & R.T.A. Cook on *Hydrangea*. *Erysiphe deutziae* has been the only powdery mildew species hitherto found on *Deutzia* spp. (Braun and Cook 2012). This species was originally described as *Microsphaera deutziae* (Bunkina 1973). In 1977, this species was recorded from the Russian Far East and Japan (Nomura 1997). Braun and Takamatsu (2000) re-allocated *M. deutziae* to *Erysiphe* based on the phylogenetic analysis of ITS rDNA sequences (Braun and Takamatsu 2000). Later, this powdery mildew was introduced to Europe with records from France, Germany, Poland and Switzerland (Bolay et al. 2005) and the UK (Denton and Henricot 2007). In recent years, this pathogen was also reported on *Deutzia* in Korea (Park et al. 2010) and China (Nguyen et al. 2018).

In 2018, leaves of *D. parviflora* with clearly dense powdery layers were collected twice. Microscopic examination suggested the unusual appendages of chasmothecia of the fungus are apparently distinct from *E. deutziae* on *Deutzia*. In order to circumscribe this species, morphological and molecular phylogenetic analyses, based on ITS and 28S rDNA sequences, were conducted for the characterisation and identification of a new *Erysiphe* species, *E. deutziicola*, found in China on *D. parviflora*.

Materials and methods

Morphological studies

In May 2018, *D. parviflora* plants with typical white powdery mildew symptoms were first noticed and collected in the nature reserve of Baihua Mountain of Beijing, China (115°34.20'E; 39°50.40'N) and later, in October, the sexual morph was found. The two specimens were deposited in the Herbarium of Mycology of Jilin Agricultural University (**HMJAU**) under the accession number HMJAU-PM91860 and HMJAU-PM91861, respectively. The dried specimens were put in lactic acid for light microscopic examinations (Zeiss Axio Scope A1, Germany).

DNA extraction and sequencing

Genome DNA was extracted using chasmothecia of HMJAU-PM91860 and conidia and mycelia from the asexual specimen HMJAU-PM91861 by the Chelex-100 method (Walsh et al. 1991; Hirata and Takamatsu 1996). Two specimens of *Erysiphe deutziae* on *Deutzia parviflora* var. *amurensis* (Nguyen et al. 2018) were also used for the DNA extraction and amplification, since 28S rDNA sequences of *E. deutziae* were not yet available in GenBank. The DNA amplification and sequencing were conducted according to the procedure described by Qiu et al. (2018).

Molecular phylogenetic analyses

The newly obtained sequence data (28S rDNA, including domains D1 and D2 and ITS, including the 5.8S rDNA) from two powdery mildew specimens on *D. parviflora* were aligned to confirm the homology. The new sequences were deposited in GenBank under accession numbers MK656288 (ITS) and MK656309 (28S) from HMJAU-PM91860 and MK656289 (ITS) and MK656310 (28S) from HMJAU-PM91861. The combined datasets of ITS and 28S rDNA sequences from the two specimens were aligned with closely related sequences of *Erysiphe* spp. retrieved from GenBank (Table 1) including sequences from some species occurring on hosts belonging to the Hydrangeaceae using MUSCLE implemented in the MEGA 7 programme (Kumar et al. 2016). Alignments were further manually refined and deposited in TreeBASE (http://www.treebase.org/) under the accession number of S24214.

Table 1. Vouchers, hosts and GenBank accession numbers of the sequences used in this study.

Species	Voucher	Host	Host family	Accession number	Sequence size (bp)	Reference
Erysiphe adunca	MUMH 171	*Salix futura*	Salicaceae	LC028968	1326	Takamatsu et al. 2015b
E. arcuata	MUMH 2741	*Carpinus tschonoskii*	Betulaceae	AB252473	1335	Braun et al. 2006
E. arcuata	MUMH 3620	*C. tschonoskii*	Betulaceae	AB252474	1335	Braun et al. 2006
E. blasti	MUMH 0002	*Laurus umbellata*	Lauraceae	LC009905	1317	Takamatsu et al. 2015a
E. deutziae	HMJAU91777	*Deutzia parviflora* var. *amurensis*	Hydrangeaceae	MH027420 (ITS)	671	Nguyen et al. 2018
				MK656311 (28S)	637	This study
E. deutziae	HMJAU91771	*D. parviflora* var. *amurensis*	Hydrangeaceae	MG674082 (ITS)	670	Nguyen et al. 2018
				MK656312 (28S)	637	This study
E. deutziicola	HMJAU-PM91860	*D. parviflora*	Hydrangeaceae	MK656288 (ITS)	666	**This study**
				MK656309 (28S)	636	
E. deutziicola	HMJAU-PM91861	*D. parviflora*	Hydrangeaceae	MK656289 (ITS)	666	**This study**
				MK656310 (28S)	636	
E. epigena	MUMH 2193	*Quercus variabilis*	Fagaceae	AB292720	1403	Takamatsu et al. 2007
E. heraclei	MUMH 2484	*Conium maculatum*	Umbelliferae	LC010021	1355	Takamatsu et al. 2015a
E. huayinensis	MUMH 4644	*Isodon umbrosus*	Lamiaceae	LC010072	1314	Takamatsu et al. 2015a
E. huayinensis	MUMH 0087	*I. trichocarpus*	Lamiaceae	LC010080	1362	Takamatsu et al. 2015a
E. hydrangeae	MUMH 0514	*Hydrangea paniculata*	Hydrangeaceae	LC028983	1361	Takamatsu et al. 2015b

E. izuensis	MUMH 4651	*Rhododendron reticulatum*	Ericaceae	LC010076	1350	Takamatsu et al. 2015a
E. juglandis	TPU 1745	*Pterocarya rhoifolia*	Juglandaceae	LC010090	1276	Takamatsu et al. 2015a
E. pileae	MUMH 2987	*Pilea pumila*	Urticaceae	LC010059 (ITS)	552	Takamatsu et al. 2015a
				LC010058 (28S)	754	
E. pedaliacearum	MUMH 412	*Sesamum indicum*	Pedaliaceae	LC342968	1516	Shin et al. 2018
E. phyllanthi	MUMH 0099	*Phyllanthus flexuosus*	Euphorbiaceae	LC009921	1351	Takamatsu et al. 2015a
E. sedi	MUMH 2576	*Sedum* sp.	Crassulaceae	LC010046	1321	
E. schizophragmatis	MUMH 4642	*Hydangea petiolaris*	Hydrangeaceae	LC029001	1356	Takamatsu et al. 2015b
Pseudoidium hortensiae	MUMH 0071	*Hydrangea macrophylla*	Hydrangeaceae	LC009915	1249	Takamatsu et al. 2015a
Pse. neolycopersici	MUMH 0066	*Lycopersicon esculentum*	Solanaceae	LC009912	1344	Takamatsu et al. 2015a

A phylogenetic tree was obtained from the combined data using the maximum-parsimony (MP) method in PAUP 4.0. The MP analysis was performed with the heuristic search option using the tree-bisection-reconstruction (TBR) algorithm with 100 random sequence additions to find the global optimum tree. The gaps were treated as missing data. The bootstrap analysis (1000 replications) was used for testing the strength of the internal branches of the resulting trees (Felsenstein 1985). Tree scores, including tree length, consistency index (CI), retention index (RI) and rescaled consistency index (RC) were also calculated. Bootstrap (BS) values of 60% or higher are indicated.

Results

Phylogenetic analysis

The alignments of ITS and 28S rDNA sequences obtained from the two specimens examined are identical to each other. A total of 22 combined sequence data, including sequences from *Pseudoidium hortensiae*, *E. hydrangeae*, *E. schizophragmatis* and *E. deutziae*, four powdery mildew species on hosts of the Hydrangeaceae, were used to construct the phylogenetic tree. The sequence of *E. adunca* (LC028968) was used as outgroup. The original alignment dataset comprises of 1232 characters. We manually deleted 111 characters and the remaining 1121 characters were finally used for constructing the phylogenetic tree, where 105 characters were variable but not informative and 175 characters were phylogenetically informative for parsimony analysis. The analysis produced three equally parsimonious trees. The best MP tree (TL = 525, CI = 0.6895, RI = 0.7175, RC = 0.4947) with the highest likelihood score is shown in Figure 1. The phylogenetic analysis confirmed that the new sequences obtained from the powdery mildew on *D. parviflora* formed an independent clade supported by a bootstrap value of 100%.

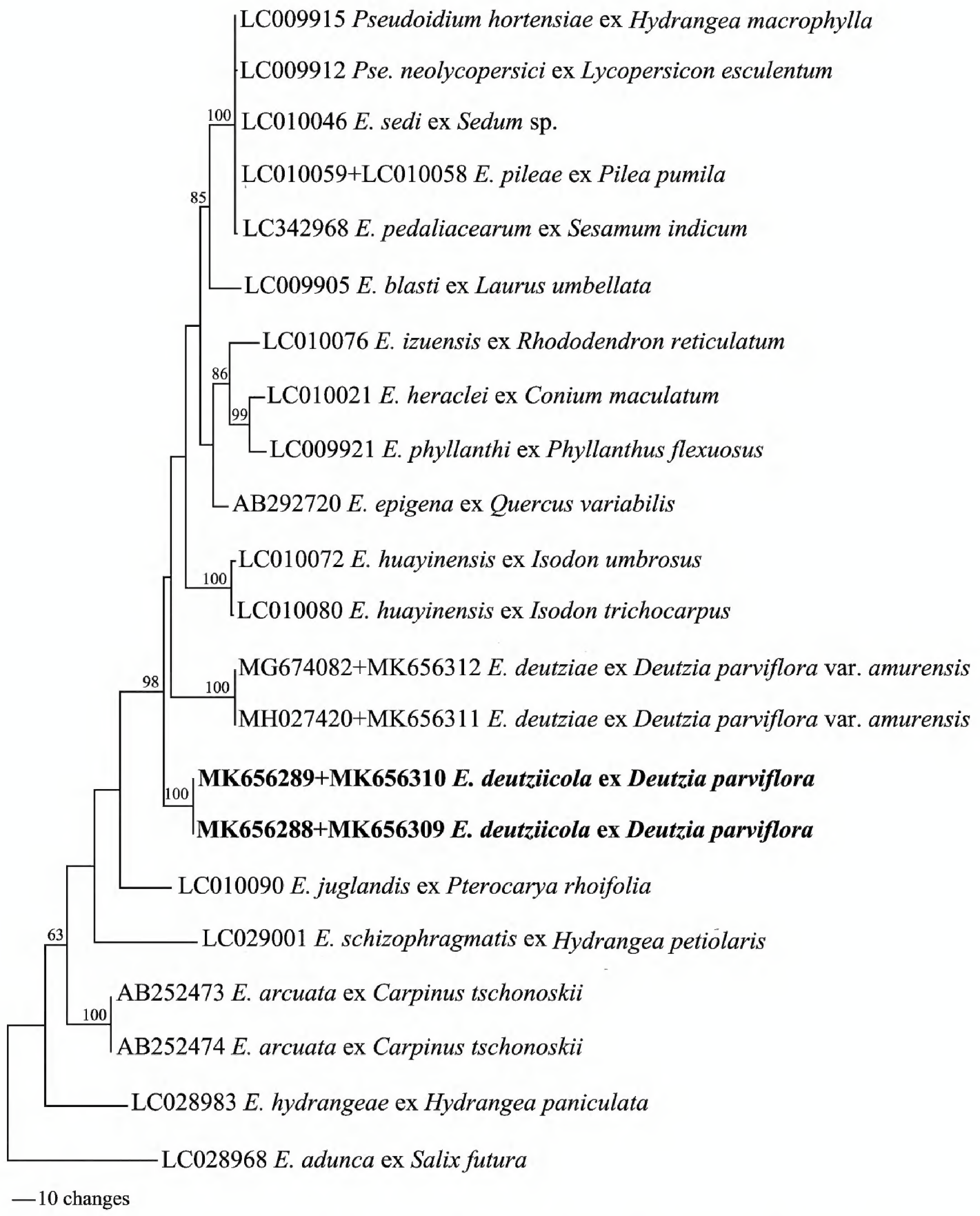
LC009915 Pseudoidium hortensiae ex Hydrangea macrophylla
LC009912 Pse. neolycopersici ex Lycopersicon esculentum
100
LC010046 E. sedi ex Sedum sp.
LC010059+LC010058 E. pileae ex Pilea pumila
85
LC342968 E. pedaliacearum ex Sesamum indicum
LC009905 E. blasti ex Laurus umbellata
LC010076 E. izuensis ex Rhododendron reticulatum
86
LC010021 E. heraclei ex Conium maculatum
99
LC009921 E. phyllanthi ex Phyllanthus flexuosus
AB292720 E. epigena ex Quercus variabilis
LC010072 E. huayinensis ex Isodon umbrosus
100
LC010080 E. huayinensis ex Isodon trichocarpus
98
MG674082+MK656312 E. deutziae ex Deutzia parviflora var. amurensis
100
MH027420+MK656311 E. deutziae ex Deutzia parviflora var. amurensis
MK656289+MK656310 E. deutziicola ex Deutzia parviflora
100
MK656288+MK656309 E. deutziicola ex Deutzia parviflora
LC010090 E. juglandis ex Pterocarya rhoifolia
63
LC029001 E. schizophragmatis ex Hydrangea petiolaris
AB252473 E. arcuata ex Carpinus tschonoskii
100
AB252474 E. arcuata ex Carpinus tschonoskii
LC028983 E. hydrangeae ex Hydrangea paniculata
LC028968 E. adunca ex Salix futura
10 changes

Figure 1. Maximum parsimony phylogram of *Erysiphe deutziicola* and its allied species constructed from the combination of ITS and 28S rDNA sequences. *Erysiphe adunca* (LC028968) was used as outgroup. Bootstrap values (> 60%) by the maximum parsimony (MP) method are shown on the respective branches. The sequences pertaining to *E. deutziicola* are shown in bold face.

Taxonomy

Erysiphe deutziicola **P.-L. Qiu, S.-Y. Liu & Y. Li, sp. nov.**
Figure 2
MycoBank: MB830253

Etymology. Named after the host genus, *Deutzia*, + -icola (dweller).

Diagnosis. Differs from all known *Erysiphe* species on hosts belonging to the Hydrangeaceae in having very long conidiophores, up to 235.0 µm and chasmothecia with mycelioid appendages, circinate at the apex.

Type. CHINA. Beijing City, Baihua Mountain, on leaves of *Deutzia parviflora*, 19 October 2018, P.-L. Qiu, S.-R. Tang & L. Liu, HMJAU-PM91860 (holotype) and HMAS 248089 (isotype) in the Herbarium Mycologicum Academiae Sinica (HMAS), Beijing; ibid., on leaves of *D. parviflora*, 26 May 2018, P.-L. Qiu, S.-R. Tang & D.-N. Jin, HMJAU-PM91861 (paratype).

Description. Forming distinct white colonies, very small and dense, covering both sides of the leaves, causing discolourations of entire leaves or even malformations. Mycelium amphigenous, effuse and persistent. Hyphal appressoria distinctly lobed, solitary (Figure 2, A). Conidiophores, short to very long, 54.5−171.0(−235) × 5.8−8.0 µm, arising from the upper surface of hyphal mother cells (Figure 2, B−D). Foot-cells straight, (23−)30.5−75.0 × 5.7−7.7 µm, followed by 1 to 3(−4) cells, 13−80 µm in length. Conidia formed singly, hyaline, ellipsoid-ovoid or oblong, 18.6−35.5 × 10−14 µm with a length/width ratio varying from 1.4−3.0(−3.3) (Figure 2, E−G). Germ tubes on the conidia with lobed apex or longitubus pattern, apex simple or somewhat swollen, produced laterally, near the middle or in perihilar position (Figure 2, H−J). Chasmothecia, amphigenous, scattered, 70−100.0 µm diam. (Figure 2, K). Peridium cells irregularly polygonal, 3.5–12.5 µm diam. (Figure 2, M). Appendages 6−14 per chasmothecium, mycelioid, hyaline, aseptate, extremely long and flexuous, 1.3−7.0 times as long as the chasmothecial diameter, up to 600 µm, 3–9 µm wide in the lower half, apices mostly sinuous-geniculate or branched, circinate at the near apex, coils relatively loose and wide (Figure 2, L). Asci 4–6 per chasmothecium, broad obovoid-saccate or clavate, short-stalked or sessile, 48−71.5 × 28.5−49.5 µm (Figure 2, N−R). Ascospores ovoid or ellipsoid, 5−8 in each ascus, 13.0−20.5 × 10.5−14.5 µm (Figure 2, S−T).

Host range and distribution. On *Deutzia parviflora* (Hydrangeaceae) in Beijing, China.

Discussion

For taxonomic purposes within the genus *Erysiphe*, the characteristics of the appendages are the most effective way to assign species to morphological, non-phylogenetic sections of *Erysiphe* that were introduced in Braun and Takamatsu (2000) and Braun and Cook (2012). Of the nine species recorded on hosts of the Hydrangeaceae, only *E. poeltii*

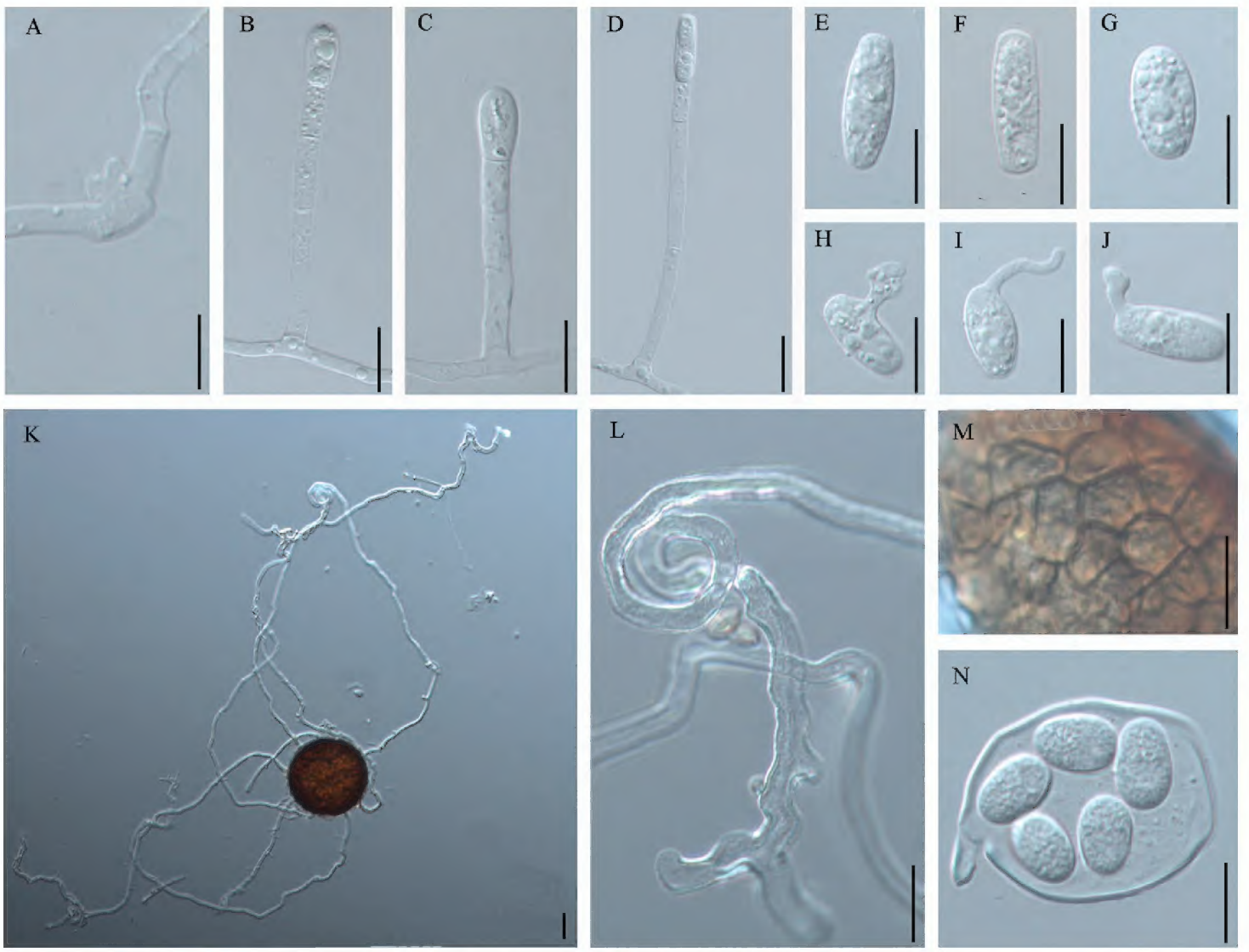

Figure 2. Morphology of *Erysiphe deutziicola* on *Deutzia parviflora*. **A** Lobed hyphal appresorium **B–D** Conidiophores **E–G** Conidia **H** Lobed germ tube arising from the lateral of conidium **I** Germ tube showing longitubus pattern arising from a conidium in perihilar position **J** Slightly lobed germ tube arising from the perihilar position of a conidium **K** Chasmothecium **L** Appendage with sinuous-geniculate, branched and circinate apex **M** Peridium cells **N** Ascus with 5 ascospores **O** Ascus with six ascospores **P** Ascus with seven ascospores **Q** Ellipsoid ascus with eight ascospores **R** Clavate ascus with eight ascospores **S** Ellipsoid ascospore **T** Ovoid ascospore. Scale bars: 20 μm.

pertains to *Erysiphe* sect. *Erysiphe* characterised by mycelioid chasmothecial appendages. The mycelioid appendages of *E. poeltii* are unbranched and later become yellowish to brownish, but remain paler or hyaline in the upper half. The appendages of *E. deutziicola* are completely hyaline, mostly sinuous-geniculate or branched in the apical region and sometimes distinctly circinate at the apex. *Erysiphe deutziae* is currently the only species on *Deutzia* spp., but it differs from *E. deutziicola* by its typically dichotomously branched appendages. The shorter, straight, stiff uncinuloid chasmothecial appendages

Table 2. Morphological comparison of *Erysiphe deutziicola* and closely related species in Braun and Cook (2012).

Species name	Host family	Conidia (µm)	Length of conidiophores (µm)	Diameter of chasmothecia (µm)	Appendages		Number of asci	Ascospores	
					Number	morphology		Number	Colour
Erysiphe deutziicola	Hydrangeaceae	18.6–35.6 × 10.2–14.1	54.7–171.0 (–234.7)	71.0–100.0	6–14	mycelioid	4–6	5–8	colourless
E. abeliae	Caprifoliaceae	– †	–	(85–) 95–120	10–40	mycelioid	4–8	6–8	yellowish
E. braunii	Asteraceae	35–45 × 17–23	80–110	90–130	18–36	mycelioid	6–16	2–3	colourless
E. deutziae	Hydrangeaceae	25–35 (–40) × (16.5–) 17.5–20 (–22)	50–75	70–150	4–16	dichotomous	2–6	4–8	colourless
E. hydrangeae	Hydrangeaceae	–	–	120–225	19–40 (–48)	circinate	(5–) 6–12 (–21)	(4–) 5–8	colourless
E. poeltii	Hydrangeaceae	26–33 × 13–18	–	75–110	5–20	mycelioid	(3–) 4–5 (–6)	5–8	colourless
E. schizophragmatis	Hydrangeaceae	27–38 × 14–18	up to 90	80–120	7–22	circinate	6–13	4–5	colourless
E. yanshanensis	Hydrangeaceae	average 26.5 × 14	45–80	average 120	5–23	circinate	(3–) 4–9 (–11)	(2–) 5–7 (–8)	yellowish
Pseudoidium hortensiae	Hydrangeaceae	(18–) 25–40 (–45) × (9–) 12–19 (–22)	40–130 (–175)	–	–	–	–	–	–

† "–" means no related information

with uncinated-circinate tips are characteristic for *E. hydrangeae*, *E. schizophragmatis* and *E. yanshanensis* and easily distinguish these species from *E. deutziicola*. Recently published phylogenetic examinations revealed that *Pseudoidium hortensiae* belongs to the *Erysiphe aquilegiae* complex (Shin et al. 2018) suggesting that *Pse. hortensiae* is a member of sect. *Erysiphe*, although chasmothecia of this species have not yet been found (Braun and Cook 2012). There are two additional species with chasmothecial appendages similar to those of *E. deutziicola*, viz., *E. abeliae* R.Y. Zheng & G.Q. Chen and *E. braunii* Y. Nomura. which have been described. However, the appendages in *E. braunii* on *Saussurea* are quite distinct by being pluriseptate and not coiled at the tip and the asci are 2–3-spored (Braun and Cook 2012). *Erysiphe deutziicola* differs from *E. abeliae* in having fewer, much longer appendages (numbers 6–14 vs. 10–40, 1.3–7.0 times as long as the chasmothecial diameter vs. mostly 1–2 times) and fewer asci (4–6 per chasmothecium vs. 4–8). In addition, the ascospores of *E. abeliae* are yellowish vs. colourless in *E. deutziicola*.

The phylogenetic analysis revealed that *Erysiphe deutziicola* clustered in a separate clade with 100% bootstrap support, distant from all included *Erysiphe* species occurring on hosts of the Hydrangeaceae and it further confirmed that this species represents a species of its own. The detail morphological traits of *E. deutziicola* and other *Erysiphe* species on hosts of the Hydrangeaceae, as well as morphologically similar species on hosts of other plant families, are shown in Table 2.

Acknowledgements

The authors sincerely acknowledge Prof. Susumu Takamatsu for reading the full text, for critical comments and for corrections. We appreciate the laboratories of Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi and College of Plant Protection in Jilin Agricultural University, Changchun, China. This work was supported by the National Natural Science Foundation of China [grant programme numbers 31670022, 31470153].

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